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The role of lipophilicity in determining binding affinity and functional activity for 5-HT_{2A} receptor ligands

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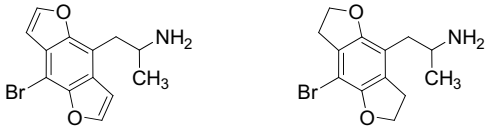
Abstract—The lipophilicity of a set of 5-HT_{2A} ligands was determined using immobilized-artificial-membrane chromatography, a method that generates values well correlated with octanol–water partition coefficients. For agonists, a highly significant linear correlation was observed between binding affinity and lipophilicity. For ligands exhibiting partial agonist or antagonist properties, the lipophilicity was consistently higher than would be expected for an agonist of comparable affinity. The results suggest a possible method for distinguishing agonists from antagonists in high-throughput screening when a direct assay for functional activity is either unavailable or impractical.

1. Introduction

The 5-HT_{2A} receptor, known to play a key role in the action of psychedelics as well as being a therapeutic target for the treatment of schizophrenia, has been a focus of SAR studies in our laboratories for many years. A particularly interesting fact that seemed to invite further investigation into the SAR of this receptor was our previously reported discovery[1] of the greatly enhanced in vitro and in vivo potency of benzodifuran agonist **1** (Fig. 1) relative to its saturated counterpart **2**.



1 K_i = 0.23 nM

2 K_i = 14.8 nM

Figure 1. Binding affinity of benzodifuran **1** and tetrahydrobenzodifuran **2** at [^{3}H]MDL 100,907-labeled rat cortical 5-HT_{2A} receptors.

The increased activity of **1**, the first non-ergoline to surpass the potency of the hallucinogen LSD in animal behavioral assays, had not been anticipated based on previous conceptions of the factors determining affinity at the 5-HT_{2A} receptor. In particular, it was difficult to imagine how the difference in activity between **1** and **2** could be explained using a 3-D receptor binding site model, as the shape of the molecule and relative positions of the heteroatoms are virtually identical for the two compounds.

One notable physical difference between **1** and **2** was observed before any pharmacological studies had been initiated: when a sample of the hydrochloride salt of **1** was being dissolved for proton NMR analysis, it went only with difficulty into D_2O, in contrast to the behavior of the HCl salt of **2**, which dissolved readily. (This difference is not entirely surprising given the fact that the solvent THF is miscible with water, whereas furan is not.) When the data revealing the high potency of **1** were later obtained, we considered three possibilities: that it was highly active due to the decreased hydrogen-bond acceptor ability of the furan oxygen atoms in **1** relative to the dihydrofuran oxygen atoms of **2** (as studies[2–4] have suggested that the ether oxygen substituents in **2** and related

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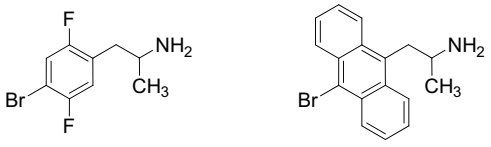
phenethylamines act as hydrogen-bond acceptors for key threonine and serine residues[5] in the 5-HT_{2A} receptor pocket) due to the extended aromaticity of the fused 3-ring system of **1** or due to the increased lipophilicity of **1**.

To investigate the first possibility, we decided to synthesize a compound in which the hydrogen-bonding capability at the position of the furan oxygen atoms is further reduced or eliminated. Fluorine has been employed as a bioisostere of oxygen, but is an extremely weak hydrogen-bond acceptor.[6,7] Therefore, the fluoro derivative **3** (Fig. 2) was chosen as a synthetic target with diminished H-bonding ability and which would present no additional bulk that might occlude side chains in the receptor pocket. To test the second possibility that the extended aromaticity of **1** was responsible for its enhanced activity, the carbon analog **4** was also selected for synthesis.

Finally, to test the third possibility and thoroughly investigate the potential role of lipophilicity in the activity of this series, we decided to measure the lipophilicity of **1**–**4** and a number of related phenethylamine type 5-HT_{2A} receptor ligands.

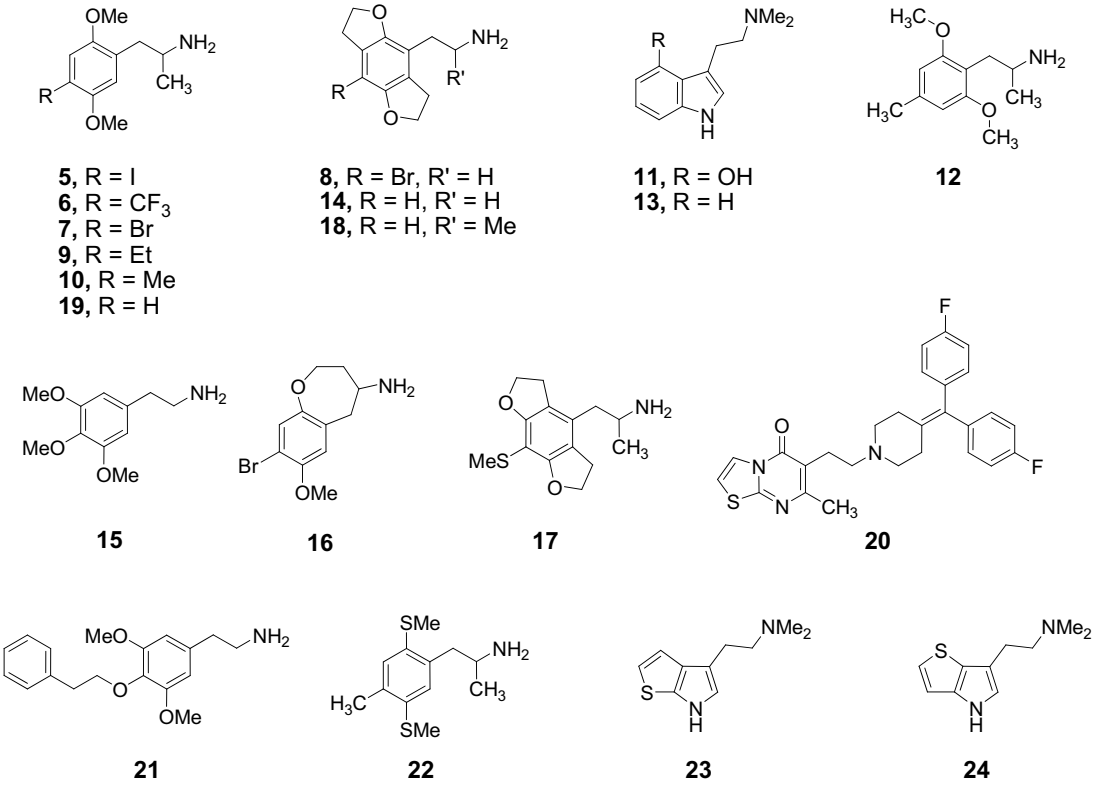


3 **4**

Figure 2. Fluoro and carbon analogs of benzodifuran **1**.

We chose immobilized-artificial-membrane (IAM) chromatography[8] as a convenient technique for quantifying the lipophilicity of **1** and **2**, as well as that of a number of comparison compounds known to be active at the 5-HT_{2A} receptor. Immobilized artificial membranes are lipids bonded to silica surfaces via a covalent link at the end of one of the constituent long-chain fatty acids. They mimic cell membranes, and capacity factors k'_{IAM} measured using IAM-packed HPLC columns correlate well with equilibrium partition coefficients of solutes partitioned between liposomes and aqueous buffer solutions. Additionally, Ong et al.[9] have reported that, for substituted phenethylamines such as a number of those examined in the current study, there is a linear relationship between the log octanol–water partition coefficient ($\log P$) and the log IAM capacity factors ($\log k'_{IAM}$) with an excellent degree of correlation ($r = 0.985$). Thus, for this set of compounds, either of these experimental techniques can be considered a valid way to quantify lipophilicity.

The set of comparison compounds in this study (Fig. 3) comprised all those that were immediately accessible in our laboratory and for which 5-HT_{2A} receptor binding and functional activity data were already available or could be readily obtained.



5, R = I
6, R = CF_3
7, R = Br
9, R = Et
10, R = Me
19, R = H

8, R = Br, R' = H
14, R = H, R' = H
18, R = H, R' = Me

11, R = OH
13, R = H

12

15 **16** **17** **20**

21 **22** **23** **24**

Figure 3. Structures of ligands **5**–**24**.

2. Results and discussion

2.1. Chemistry

All chiral compounds discussed in the current work, either new or previously reported, were synthesized and tested as racemates.

The synthesis of fluorinated analog **3** is depicted in Scheme 1. The chemistry is relatively straightforward, utilizing Henry condensation of 2,5-difluorobenzaldehyde with nitroethane, followed by reduction, protection, bromination, and finally deprotection to afford the target compound. The bromination of **27** is noteworthy, as the two fluorine atoms were found to substantially deactivate the ring toward electrophilic substitution. It was therefore necessary to employ the highly reactive conditions described by Derbyshire et al.,[10] in which deactivated arenes were brominated in good yields using a mixture of sulfuric acid, water, bromine, and silver sulfate.

The anthracene derivative **4** was prepared in similar fashion, as shown in Scheme 2. The initial condensation of 9-anthraldehyde with nitroethane proved problematic employing the usual ammonium acetate catalyst; yields of the desired nitropropene **29** were no better than 40%. Spectral data (proton NMR) of the major side product, which accounted for almost all the material not converted to **29**, were consistent with a mixture of stereoisomers of the nitroimine in Figure 4.

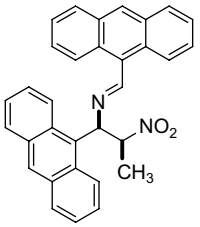
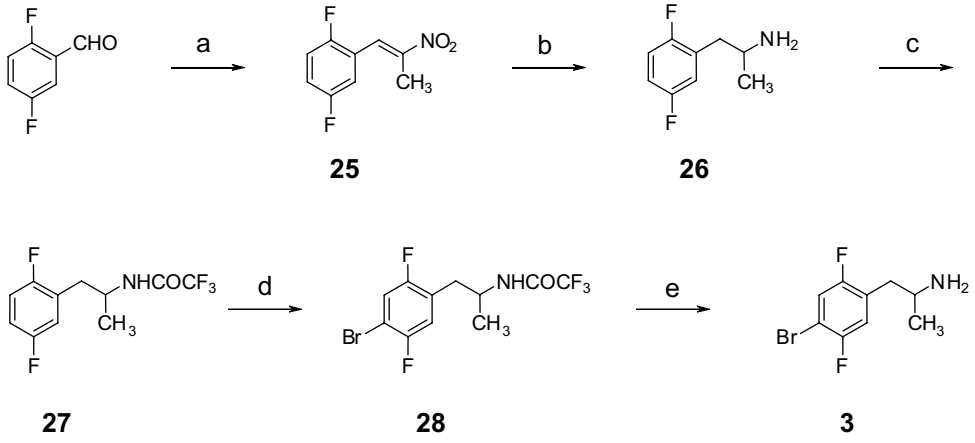
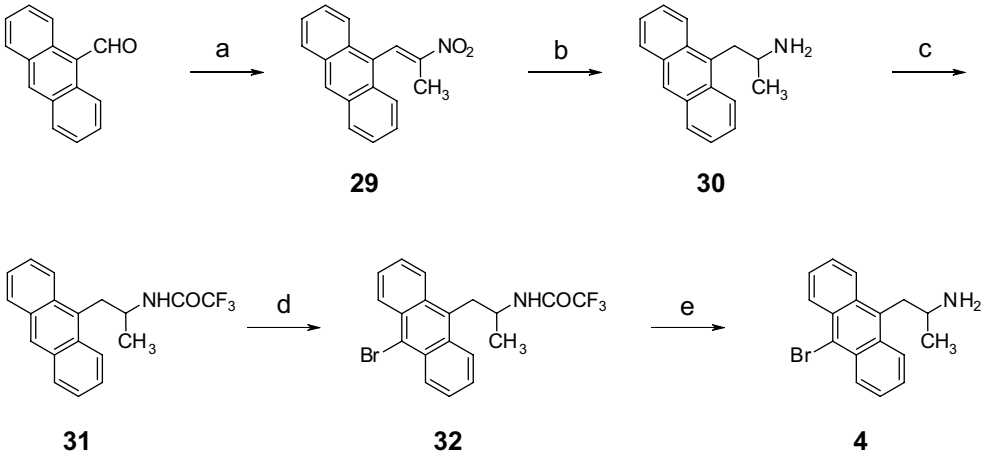


Figure 4. Nitroimine side product observed in NH_4OAc-catalyzed Henry condensation of 9-anthraldehyde and nitroethane.



Scheme 1. Synthesis of fluorinated phenethylamine derivative **3**.[a] [a]Reagents and conditions: (a) $EtNO_2$, *c*-hexNH_2, HOAc, 91%; (b) $NaBH_4$, BF_3·OEt, 77%; (c) $(CF_3CO)_2O$, Et_3N, 66%; (d) Br_2, Ag_2SO_4, H_2SO_4, 44%; (e) NaOH, H_2O/MeOH, 50%.



Scheme 2. Synthesis of anthracene derivative **4**.[a] [a]Reagents and conditions: (a) $EtNO_2$, piperidinium acetate, 97%; (b) $LiAlH_4$, 47%; (c) $(CF_3CO)_2O$, Et_3N, 33%; (d) Bu_4NBr_3, HOAc, 82%; (e) NaOH, H_2O/MeOH, 92%.

This product is analogous to an imine observed by Shulgin[11] as a major side product in the condensation of nitropropane with 3,4,5-trimethoxybenzaldehyde. Shulgin's solution to the problem, replacing the ammonium catalyst with a salt of a secondary amine, also worked in this case, increasing the yield of **29** to 97%.

Reduction with lithium aluminum hydride followed by the usual protection, bromination, and deprotection steps completed the synthesis of **4**.

2.2. Receptor binding and functional activity

Previously published K_i values for the ability of the compounds to displace the 5-HT_{2A} antagonist [^{3}H]ketanserin from rat cortical homogenates were used when available. We chose to use data from antagonist-labeled receptors in order to maximize the size of our data set, as more antagonist displacement data were available for 5-HT_{2A} ligands than agonist displacement data. Although it would have been preferable to test all compounds at the same time and with the same radioligand, for practical reasons we chose to use previously published data, although much of it originated in our laboratory, where available. The remaining compounds were assayed against the antagonist [^{3}H]MDL 100,907,[12] again in rat cortical homogenates. Functional activity of test compounds at the 5-HT_{2A} receptor, where not previously known, was determined indirectly using a drug discrimination assay in rats trained to distinguish the 5-HT_{2A} agonist LSD from saline; agonists at the 5-HT_{2A} receptor generally substitute for LSD.[13] Compounds exhibiting partial substitution in this assay are termed 'partial agonists' here, although it is difficult to distinguish a lack of full efficacy at the receptor from possible disruption of the behavioral assay due to activation of other receptor subtypes. Data are shown in Table 1.

2.3. Requirements for receptor activation

We found that fluoro analog **3** and especially carbon analog **4** bind with reasonable affinity to the 5-HT_{2A} receptor, but the drug discrimination data reveal that they lack agonist activity. Thus, the oxygen substituents of **1** and **2** appear to be required for efficacy in the phen-

Table 1. Binding affinity at antagonist-labeled 5-HT_{2A} receptors, lipophilicity, efficacy, and affinity-normalized lipophilicity for **1**–**4** and selected comparison compounds **5**–**24**

Compound (code/name)	K_i (nM) ketanserin	K_i (nM) MDL 100,907	Lipophilicity (IAM.PC α)	Efficacy*	Affinity-normalized lipophilicity, pL_n
1, BDFLY		0.23 ±0.03†	5.188	A	−0.15
2, BFLY	18 ± 1[a]	14.8 ± 1.6†	0.893	A	−0.08
5, DOI	19[b]		0.965	A	0.00
6, DOTFM	33.0 ± 5.0[c]		1.009	A	0.13
7, DOB	22 ± 3[a]; 41 ± 5[b]		0.608	A	−0.04
8, 2CBFLY	34 ± 2[a]		0.667	A	−0.04
9, DOEt	100[b]		0.678	A	0.18
10, DOM	100[b]		0.296	A	−0.18
11, Psilocin		323†	0.191	A	−0.13
12, Z7[d]	351 ± 47†		0.312	A	0.10
13, DMT		1636†	0.262	A	0.33
14, 2CHFLY	2300 ± 170[a]		0.118	A	0.05
15, Mescaline	5500 ± 600[e]		0.048	A	−0.16
16, BBOX	422 ± 16[f]		1.116	B	0.69
17, SBat[g]		476 ± 189†	0.373	B	0.24
18, HFLY	2010 ± 83[a]		0.162	B	0.16
19, 2,5-DMA	5200[b]		0.116	B	0.21
20, Ritanserin	1.7 ± 0.2[h]	36 ± 6[h]	40.233	C	1.14
21, PE[d]	59 ± 2†		1.887	C	0.52
22, BisTOM[d]	177 ± 26†		2.260	C	0.82
4, ANTH		313 ± 106†	80.076	C	2.48
3, BDFA		2505 ± 441†	0.808	C	0.90
23, T70[i]		3676†	0.154	C	0.26
24, T72[i]		4611†	0.147	C	0.28

[a] Monte (1996).[3]
[b] Seggel (1990).[19]
[c] Nichols (1994).[22]
[d] Shulgin (1991).[23]
[e] Monte (1997).[24]
[f] Monte (1995).[25]
[g] Parker (1998).[26]
[h] Johnson et al. (1996).[12]
[i] Blair (1999).[13]
* Determined indirectly using animal bioassay: A, agonist; B, partial agonist; C, antagonist.
† Previously unpublished data.

ethylamine series, and neither the extremely weak hydrogen-bonding capability of the fluoro substituents of **3** nor the extended aromaticity of **4** is sufficient for receptor activation.

2.4. Lipophilicity measurements

Lipophilicity was determined by HPLC using an immobilized-artificial-membrane column containing phosphatidylcholine head groups (IAM.PC). The experimental techniques employed and the characteristics of the IAM.PC surface have been described in detail.[8,9,14–16]

Measured IAM.PC α values, which are the capacity factors k'_{IAM} divided by the capacity factor of a standard compound, are given in Table 1.

2.5. Correlation of agonist lipophilicity with binding affinity

When the log-binding affinity at antagonist-labeled 5-HT_{2A} receptors is plotted against the log IAM.PC α (corrected capacity factor), the graph shown in Figure 5 results. Receptor affinity and lipophilicity increase moving up and to the right, respectively. The data points for compounds classified as agonists lie very nearly in a straight line ($r^2 = 0.92$). All the antagonists and partial agonists lie to the right of the line, with many of the antagonists lying far to the right.

The excellent correlation obtained in this graph validates our initial conjecture that the high potency of **1** was related to its increased lipophilicity (compound **1** is the dark square nearest the line in the upper right corner). The graph also indicates clearly that for those compounds that are agonists, the relationship between lipophilicity and binding affinity is linear.

We note that this high correlation of $r^2 = 0.92$ was obtained despite the fact that some data were obtained at different times and with different radioligand antagonists, indicating that any random error introduced must have been relatively small. (In future investigations, we plan to determine whether this correlation can be further improved by eliminating these possible sources of variance.)

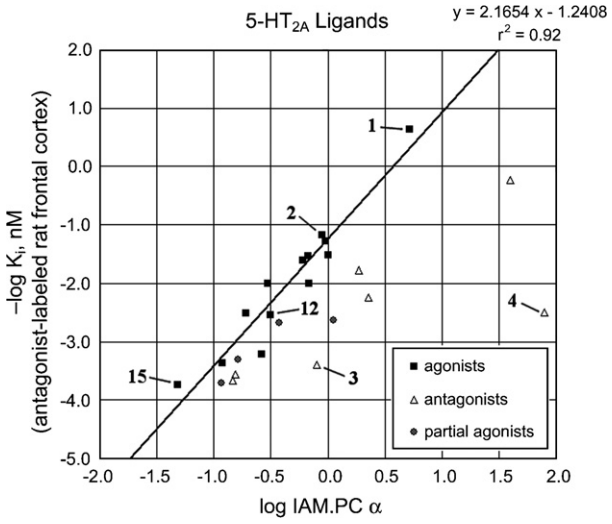


Figure 5. Log affinity at antagonist-labeled 5-HT_{2A} receptors versus log IAM.PC α.

These data provide new insight into the relationship between lipophilicity and potency in this series. Barfknecht et al.[17] observed in 1975 an apparent parabolic relation between log *P* and log human in vivo potency for a series of hallucinogenic phenethylamines, with an optimal log *P* of 3.14. The drop in activity with increasing lipophilicity occurred as the 4-alkyl substituent in 4-alkyl-2,5-dimethoxyamphetamines was lengthened. Indeed, in a 1977 study of the activity of phenethylamines in stimulating serotonin receptors in sheep umbilical artery, Nichols et al.[18] speculated that the observed drop in activity with increasing log *P* 'might be due to excessive steric bulk in the *para* position, rather than to increased hydrophobic character per se', and noted that there appeared to be a sharp limit (5–6 Å) on the length of the *para* substituent in active compounds. Thirteen years later, Seggel et al.[19] showed that this decrease in activity was due not to a lack of affinity (the 4-hexyl compound exhibited a K_i of 2.5 nM for ketanserin-labeled 5-HT_2 receptors) but to a lack of efficacy: compounds with long 4-alkyl substituents appeared to be acting as antagonists.

Now that a wider range of agonists, including the exceptionally lipophilic **1**, have been examined in the current study, it is clear that for agonists the relationship between lipophilicity (log *P* or k'_{IAM}) and log in vitro affinity is linear. The log *P* of compound **1**, calculated from the measured k'_{IAM} using the linear relationship mentioned earlier, is approximately 4.09, significantly higher than 3.14 previously believed to be optimal.

Thus, it would appear that aromatization of **2** to **1** diminishes the strength of hydrogen bonds to the furan oxygen[20] (but not to such a degree that the receptor-activating serine residues fail to form H-bonds as with **3**) and thereby dramatically increases the lipophilicity of the molecule, resulting in a very active compound.

2.6. Lipophilicity at isoaffinity as a potential correlate of functional activity

A key observation from Figure 5 is that the compounds that lack agonist activity in the rat behavioral model, or that are known to be antagonists, lie well to the right of the agonist line, and those that show only partial agonist activity lie closer to the line but still trend to the right of it. In other words, each antagonist is more lipophilic than an agonist with the same affinity would be.

This phenomenon can be quantified by defining a value L_n, the affinity-normalized lipophilicity, as the lipophilicity of the ligand in question divided by the lipophilicity that would be expected for an agonist with the same affinity as determined by the line previously fit to the agonist data. Then the logarithmic form of the parameter, $pL_n = -\log L_n$, becomes a convenient descriptor of

how many log units to the left or right of the agonist line a given point lies on a log–log plot such as Figure 5.

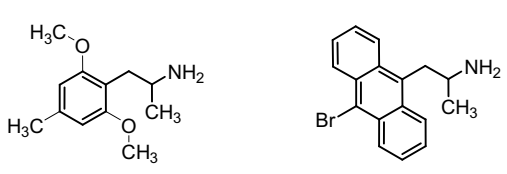
It stands to reason that agonists acquire a greater portion of their binding affinity through interaction with relatively hydrophilic amino acid side chains in the receptor pocket than do antagonists. Antagonists in general bind to a greater degree through hydrophobic interactions with the receptor and by definition do not exert critical conformation-changing binding forces on amino acid residues inside the pocket. Thus, in order to have the same affinity as a given agonist, they must generally be more lipophilic than that agonist. The pL_n metric quantifies this property and thereby provides an indication of agonist/antagonist properties of ligands if a line for agonists on the affinity–lipophilicity plot has already been determined. A pL_n value close to zero indicates that the ligand is likely to be an agonist, and a pL_n value greater than about 0.5 indicates the opposite. In the current data set, 12 of the 13 agonists, and none of the antagonists, have pL_n values less than 0.20 (Table 1). Moreover, five of the seven antagonists have pL_n values greater than 0.50. The partial agonists tend to lie in between these two limits.

In the current data set, comparison of agonist **12** and antagonist **4** is particularly instructive (Fig. 6). They have nearly identical affinity for the 5-HT_{2A} receptor (313 nM vs 351 nM) but **4** is ~2.5 log units more lipophilic than **12**. Thus, it binds to the receptor by virtue of its lipophilicity and lacks appropriate functional groups, such as the 2- and 6-alkoxy substituents of **12**, to activate the receptor.

We should emphasize that the data do not support the conclusion that antagonists are on average more lipophilic than agonists; for example, antagonist **3** is far less lipophilic than agonist **1**. Rather, it is the *affinity-normalized lipophilicity* pL_n, calculable only after a function relating lipophilicity to binding affinity for a set of agonists has been determined, that appears to be an indicator of functional activity.

3. Conclusions

The data support the conclusion that the enhanced potency of **1** over **2** is due to the fact that the lipophilicity is increased while key elements of the 5-HT_{2A} pharmacophore (the furan oxygen substituents) are retained.



12 K_i = 351 nM

4 K_i = 313 nM

Figure 6. Binding affinity of agonist **12** and antagonist **4** at antagonist-labeled rat cortical 5-HT_{2A} receptors.

For the full set of 5-HT_{2A} receptor ligands examined, the correlation between lipophilicity and binding affinity is a good linear fit for agonists. This correlation allows the specification of a new parameter, the affinity-normalized lipophilicity L_n, as a potential indicator of agonist versus antagonist activity. Further work is planned to verify the current findings by increasing the size of the data set as well as measuring efficacy directly by second-messenger detection; we also plan to explore the scope of the method for additional 5-HT_{2A} ligand chemotypes and determine whether it can be extended to other monoamine receptors. If so, it may prove useful in high-throughput screening, allowing agonists to be distinguished from antagonists in cases where functional activity of ligands cannot easily be measured directly.

4. Experimental

4.1. Chemistry

All reagents were commercially available and were used without further purification unless otherwise indicated. Anhydrous THF was obtained by distillation from benzophenone–sodium under nitrogen. Melting points were taken on a Mel-Temp apparatus and are uncorrected. ^{1}H NMR spectra were recorded using a 500 MHz Varian VXR-500S spectrometer. Chemical shifts are reported in δ values ppm relative to tetramethylsilane as an internal reference for those spectra obtained in $CDCl_3$, and relative to the HDO resonance, assigned the value of 4.630 ppm, for those spectra obtained in D_2O. Elemental analyses were performed by H.D. Lee at the Purdue University Microanalysis Laboratory and are within 0.4% (absolute) of the calculated values. Most reactions were carried out under an inert atmosphere of dry argon or nitrogen.

4.1.1. 1-(2,5-Difluorophenyl)-2-nitropropene (25). A mixture of 2.0 g (14 mmol) of 2,5-difluorobenzaldehyde, 2.2 g (29 mmol) of nitroethane, and 1.4 g (14 mmol) of cyclohexylamine in 12 mL of acetic acid was heated and stirred at 80 °C under argon for 5 h. The mixture was cooled to room temperature, poured into 200 mL of water, and extracted 3× with CH_2Cl_2. The pooled extracts were washed twice with water and once with 5% aqueous NaOH. The resulting solution was dried with $MgSO_4$, filtered, and evaporated to leave 2.55 g (91%) of **25** as a clear yellow oil which crystallized on standing overnight under vacuum. An analytical sample gave light yellow plates from hexane: mp 48–49.5 °C. ^{1}H NMR ($CDCl_3$) δ 2.39 (s, 3, CH_3), 7.08 (m, 1, ArH), 7.14 (m, 2, ArH), 8.03 (s, 1, vinylic H). Anal. Calcd for $C_9H_7F_2NO_2$: C, 54.28; H, 3.54; N, 7.03. Found: C, 54.01; H, 3.61; N, 6.93.

4.1.2. 1-(2,5-Difluorophenyl)-2-aminopropane (26). In a dry round-bottomed flask were placed 2.51 g of sodium borohydride and 105 mL of dry THF. The flask was cooled to 0 °C under argon and 10.4 mL of boron trifluoride diethyl etherate was added via syringe. After the addition, the flask was warmed to room temperature and stirred for 15 min. A solution of 2.77 g of **25** in

35 mL of THF was added dropwise via syringe, and the reaction mixture was heated at reflux for 5.5 h. The mixture was cooled to room temperature, quenched carefully by the (initially dropwise) addition of water (175 mL), acidified with 90 mL of 2 M HCl, and heated at 80–85 °C for 2 h. After cooling to room temperature, most of the THF was removed under reduced pressure and the remaining material was washed once with Et_2O. The aqueous layer was separated, made strongly alkaline with 10% aqueous NaOH, and extracted three times with Et_2O. The Et_2O extracts were dried with $MgSO_4$, filtered, and evaporated, yielding 1.83 g (77%) of the crude amine **26** as a yellow oil. ^1H NMR ($CDCl_3$) δ 1.13 (d, 3, CH_3, $J = 6.4$ Hz), 1.88 (br s, 2, NH_2), 2.60 (dd, 1, $ArCH_2$, $J = 7.7$, 13.3 Hz), 2.71 (dd, 1, $ArCH_2$, $J = 5.7$, 13.3 Hz), 3.21 (sextet, 1, $ArCH_2CH$, $J = 6.3$ Hz), 6.86–6.93 (complex m, 2, ArH), 6.98 (dt, 1, ArH, $J = 4.5$, 8.9 Hz). The amine was converted to the trifluoroacetamide **58** without further characterization.

4.1.3. *N*-Trifluoroacetyl-1-(2,5-difluorophenyl)-2-aminopropane (27). To a solution of 1.3 g (7.6 mmol) of crude**26** in 100 mL of CH_2Cl_2 under argon was added 1.26 mL (9.13 mmol) of triethylamine. A solution of 3.27 mL (23.2 mmol) of trifluoroacetic anhydride in 10 mL of CH_2Cl_2 was added dropwise via syringe with stirring. The solution was stirred at room temperature for 15 min, and the volatiles were removed under reduced pressure. The residue was partitioned between Et_2O and water. The Et_2O phase was dried with $MgSO_4$, filtered, and evaporated, and the residue (2.06 g) was recrystallized from EtOAc–hexane, yielding 1.33 g (66%) of **27** as beige crystals: mp 90–92 °C. ^1H NMR ($CDCl_3$) δ 1.27 (d, 3, CH_3, $J = 6.8$ Hz), 2.87 (d, 2, $ArCH_2$, $J = 6.9$ Hz), 4.29 (septet, 1, $ArCH_2CH$, 7.1 Hz), 6.23 (br s, 1, NH), 6.87–6.95 (complex m, 2, ArH), 7.02 (dt, 1, ArH, $J = 4.4$, 9.3 Hz). Anal. Calcd for $C_{11}H_{10}F_5NO$: C, 49.45; H, 3.77; N, 5.24. Found: C, 49.17; H, 3.52; N, 5.01.

4.1.4. *N*-Trifluoroacetyl-1-(4-bromo-2,5-difluorophenyl)-2-aminopropane (28). To 3.66 g of water in a 100 mL round-bottomed flask was added 41.6 g of 98% sulfuric acid. To the warm stirred solution was added 1.22 g of crystalline **27**. When all the solid had dissolved, the flask was cooled to room temperature, and 0.76 g of elemental bromine was added, followed by 0.76 g of silver sulfate. The mixture was stirred 1 h at room temperature and then poured carefully over ice. The quenched mixture was partitioned between Et_2O and H_2O, and the Et_2O phase was washed three times with H_2O and twice (carefully) with saturated aqueous $NaHCO_3$, dried with $MgSO_4$, filtered, and evaporated. The solid residue (2.35 g) was dissolved in 1.2 mL of hot EtOAc and diluted with 160 mL of warm hexane. On cooling, 0.694 g (44%) of **28** precipitated as white crystals: mp 128–130 °C. ^1H NMR ($CDCl_3$) δ 1.27 (d, 3, CH_3, $J = 6.7$ Hz), 2.85 (d, 2, $ArCH_2$, $J = 6.7$ Hz), 4.28 (septet, 1, $ArCH_2CH$, $J = 7.1$ Hz), 6.13 (br s, 1, NH), 6.97 (dd, 1, ArH, $J = 6.2$, 8.3 Hz), 7.29 (dd, 1, ArH, $J = 5.6$, 8.7 Hz). Anal. Calcd for $C_{11}H_9BrF_5NO$: C, 38.17; H, 2.62; N, 4.05. Found: C, 37.99; H, 2.49; N, 3.82.

4.1.5. 1-(4-Bromo-2,5-difluorophenyl)-2-aminopropane hydrochloride (3). A solution of 2 g of NaOH in 10 mL of H_2O was added to a solution of 0.500 g of amide **28** in 40 mL of methanol. The mixture was stirred under argon for 24 h at room temperature. The methanol was removed by rotary evaporation and the remaining mixture was partitioned between Et_2O and H_2O. The Et_2O phase was washed with H_2O, dried with $MgSO_4$, filtered, and evaporated. The residue was dissolved in 2 mL of EtOH and acidified with 1 mL of 2 M anhydrous HCl in ethanol. Dilution with Et_2O produced a fine white precipitate of 0.205 g (50%) of the hydrochloride salt: mp 193–194 °C. ^1H NMR (D_2O) δ 1.12 (d, 3, CH_3, $J = 6.7$ Hz), 2.75 (dd, 1, $ArCH_2$, $J = 7.3$, 14.2 Hz), 2.81 (dd, 1, $ArCH_2$, $J = 6.6$, 14.2 Hz), 3.47 (sextet, 1, $ArCH_2CH$, $J = 6.8$ Hz), 7.04 (dd, 1, ArH, $J = 6.4$, 8.7 Hz), 7.31 (dd, 1, ArH, $J = 5.8$, 9.0 Hz). Anal. Calcd for $C_9H_{11}BrClF_2N$: C, 37.72; H, 3.87; N, 4.89. Found: C, 37.63; H, 3.69; N, 4.68.

4.1.6. 1-(9-Anthracenyl)-2-nitropropene (29). A mixture of 4.0 g of 9-anthraldehyde, 33 mL of nitroethane, and 2.81 g of piperidinium acetate in a round-bottomed flask under an argon atmosphere was placed into an oil bath already heated to 90 °C and stirred for exactly 30 min. The flask was removed from the oil bath and cooled under running water, then poured into 200 mL water. The mixture was extracted twice with CH_2Cl_2. The organic phases were combined, washed with water, dried with $MgSO_4$, filtered, and evaporated, yielding 4.95 g (97%) of **29** as bright orange crystals, quite clean by proton NMR. An analytical sample was recrystallized from THF/hexane: mp 142–142.5 °C. ^1H NMR ($CDCl_3$) δ 2.01 (s, 3, CH_3), 7.54 (pentet, 4, ArH, $J = 7$ Hz), 7.90 (d, 2, ArH, $J = 8.5$ Hz), 8.07 (d, 2, ArH, $J = 7.5$ Hz), 8.54 (s, 1, ArH), 8.79 (s, 1, vinylic H). Anal. Calcd for $C_{17}H_{13}NO_2$: C, 77.55; H, 4.98; N, 5.32. Found: C, 77.35; H, 4.76; N, 5.15.

4.1.7. 1-(9-Anthracenyl)-2-aminopropane (30). A solution of 4.55 g of **29** in 160 mL of anhydrous THF was added dropwise under argon to a stirred suspension of 4.00 g of $LiAlH_4$ in 160 mL of anhydrous THF. The mixture was stirred and heated at reflux for 42 h. The mixture was cooled to room temperature and slowly quenched, sequentially, with 4.5 mL of 2-propanol, 4.5 mL of 15% aqueous NaOH, and 16 mL of water. The resulting solids were filtered off and the filtrate was evaporated. The residue was partitioned between 200 mL of Et_2O and 200 mL of 0.5 M HCl. The aqueous phase was washed twice with Et_2O and basified with aqueous NaOH, then extracted 3× with Et_2O. The Et_2O extracts were dried with $MgSO_4$, filtered, and evaporated, yielding 1.90 g (47%) of the crude free amine **30** as a viscous tan oil. ^1H NMR ($CDCl_3$) δ 1.28 (d, 3, CH_3, $J = 6.2$ Hz), 3.54 (sextet, 1, $ArCH_2CH$, 6.7 Hz), 3.66 (dd, 1, $ArCH_2$, $J = 7.6$, 14.2 Hz), 3.71 (dd, 1, $ArCH_2$, $J = 6.0$, 14.2 Hz), 7.47 (t, 2, ArH, $J = 7.5$ Hz), 7.51 (t, 2, ArH, $J = 7.5$ Hz), 8.01 (d, 2, ArH, $J = 8$ Hz), 8.33 (d, 2, ArH, $J = 9$ Hz), 8.37 (s, 1, ArH). This product

was converted to the trifluoroacetamide derivative **31** without further characterization.

4.1.8. *N*-Trifluoroacetyl-1-(9-anthracenyl)-2-aminopropane (31). A solution of 1.88 g (8 mmol) of the crude amine **30** and 1.12 mL (0.810 g, 8 mmol) of triethylamine in 100 mL of dichloromethane was placed under argon and cooled to 0 °C in an ice bath. Trifluoroacetic anhydride (2.26 mL, 16 mmol) was added dropwise via syringe with stirring. The ice bath was removed and the solution was stirred for another 2 h, after which the reaction mixture was worked up as described previously. Recrystallization of the crude material from EtOAc/hexane gave 0.869 g (33%) of **31** as a yellow crystalline solid: mp 203–204 °C. ^{1}H NMR ($CDCl_3$) δ 1.21 (d, 3, CH_3, J = 7 Hz), 3.78 (dd, 1, $ArCH_2$, J = 9, 14 Hz), 4.07 (dd, 1, $ArCH_2$, J = 5.5, 14 Hz), 4.54 (broad septet, 1, $ArCH_2CH$, J = 8 Hz), 6.36 (br s, 1, NH), 7.48 (t, 2, ArH, J = 7.5 Hz), 7.57 (t, 2, ArH, J = 7.5 Hz), 8.03 (d, 2, ArH, J = 8.5 Hz), 8.39 (d, 2, ArH, J = 9 Hz), 8.42 (s, 1, ArH). Anal. Calcd for $C_{19}H_{16}F_3NO$: C, 68.88; H, 4.87; N, 4.23. Found: C, 68.82; H, 4.76; N, 4.08.

4.1.9. *N*-Trifluoroacetyl-1-(10-bromoanthracen-9-yl)-2-aminopropane (32). A solution of 0.500 g of **31** in 20 mL warm acetic acid was cooled to room temperature and 0.786 g tetrabutylammonium tribromide was added. The mixture was stirred at room temperature for 2 h. The reaction mixture was partitioned between Et_2O and H_2O. The Et_2O phase was separated, washed with H_2O and brine, dried with $MgSO_4$, filtered, and evaporated. The solid yellow residue was recrystallized from EtOAc/hexane to give 0.507 g (82%) of **32** as a fine yellow crystalline solid: mp 215–216 °C. ^{1}H NMR ($CDCl_3$) δ 1.19 (d, 3, CH_3, J = 6.5 Hz), 3.77 (dd, 1, $ArCH_2$, J = 9.5, 14.5 Hz), 4.10 (dd, 1, $ArCH_2$, J = 5.5, 14.5 Hz), 4.52 (broad septet, 1, $ArCH_2CH$, J = 7 Hz), 6.36 (br s, 1, NH), 7.62 (m, 4, ArH), 8.45 (br s, 2, ArH), 8.64 (m, 2, ArH). Anal. Calcd for $C_{19}H_{15}BrF_3NO$: C, 55.63; H, 3.69; N, 3.41. Found: C, 55.65; H, 3.48; N, 3.19.

4.1.10. 1-(10-Bromoanthracen-9-yl)-2-aminopropane hydrochloride (4). A solution of 2.4 g of NaOH in 12 mL of H_2O was added to a solution of 0.350 g of amide **32** in 60 mL of methanol. The mixture was stirred under argon for 24 h at room temperature. The methanol was removed by rotary evaporation and the remaining mixture was partitioned between Et_2O and H_2O. The Et_2O phase was washed with H_2O, dried with $MgSO_4$, filtered, and evaporated, leaving the free amine as a yellow solid. The solid was dissolved in 6 mL EtOH and neutralized with 2 M anhydrous HCl in ethanol. Et_2O (100 mL) was slowly added. The hydrochloride salt precipitated and was collected by filtration and washed with Et_2O. Yield 0.276 g (92%) of the hydrochloride salt as fine lemon-yellow crystals: mp > 290 °C (decomp.) ^{1}H NMR (1:1 v/v DMSO-d_6/D_2O) δ 1.11 (d, 3, CH_3, J = 6.4 Hz), 3.76 (sextet, 1, $ArCH_2CH$, J = 7.6 Hz), 3.98 (m, 2, $ArCH_2$), 7.75 (m, 4, ArH), 8.42 (d, 2, ArH, J = 7 Hz), 8.60 (d, 2, ArH, J = 7 Hz). Anal. Calcd for $C_{17}H_{17}BrClN$: C, 58.23; H, 4.89; N, 3.99. Found: C, 58.28; H, 4.88; N, 3.87.

4.2. Pharmacology—radioligand competition assays in rat brain homogenate

The procedure of Johnson et al.[21] was employed with minor modifications. Briefly, 50 male Sprague–Dawley whole rat brains (unstripped) were purchased from Harlan Bioproducts for Science, Inc. and dissected over dry ice. The frontal cortex tissue was homogenized (Kinematica Polytron, setting '4', 2× 20 s) in four volumes (w/v) of ice-cold 0.32 M sucrose and centrifuged at 36,000*g* for 10 min at 4 °C. The pellet was again suspended in the same volume of sucrose, homogenized (Kinematica Polytron, setting '4', 20 s), separated into aliquots of 4.5 mL, and stored at −70 °C.

For each experiment one aliquot of frontal cortex tissue was thawed and diluted with 25 volumes (w/v) of 50 mM Tris(hydroxymethyl)aminomethane (Aldrich Chemicals) buffer, adjusted to pH 7.4 by HCl. The tissues were homogenized (Kinematica Polytron, setting '4', 20 s) and incubated for 10 min at 37 °C in a shaking water bath. The homogenate was then centrifuged twice at 36,000*g* at 4 °C for 10 min, with the pellet being resuspended in 25 volumes of Tris–HCl buffer in between. The supernatant was discarded and the pellet was resuspended with 25 volumes of Te Pac buffer (0.5 mM Na_2EDTA, 10.0 μM pargyline, 5.7 mM $CaCl_2$, 0.1% Na_2ascorbate), homogenized using the Kinematica as above, and incubated for 10 min at 37 °C in a shaking water bath. The homogenate was then placed in an ice bath to cool. Binding was initiated by adding 800 μL of homogenate tissue to assay tubes containing 100 μL of [^{3}H]MDL 100,907 (0.2 nM) and 100 μL of the competing drug solution or H_2O. Non-specific binding was determined in the presence of cinanserin (10 μM). Binding assays were incubated for 15 min at 37 °C in a shaking water bath. Incubation was stopped by rapid vacuum filtration through GF/C filters using a Brandel Cell Harvester (Brandel Instruments, Gaithersburg, MD, USA). The filters were washed twice with 5 mL aliquots of ice-cold Tris–HCl buffer, allowed to air-dry and placed into scintillation vials containing 10 mL of Ecolite scintillation cocktail (ICN Biomedicals). Eight hours later the radioactivity was measured using liquid scintillation spectroscopy (Packard model 4430) at 37% efficiency. EC_{50} (nM) values were calculated from at least three experiments, each done in triplicate, by using GraphPad PRISM™.

4.3. Immobilized-artificial-membrane chromatography

The retention times (t_R, in min) were measured on an IAM.PC.DD column (Regis Technologies, Morton Grove, IL) using a mobile phase of 10 mM PBS buffered at pH 7.4. k'_{IAM} was calculated from $k'_{IAM} = (t_R - t_0)/t_0$, where t_0 is the retention time of an unretained compound, that is, citric acid.

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